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MEMORANDUM

SUBJECT: Attached Data Evaluation Record, Chlorpyrifos: Two-Year Dietary Exposure Studies in Beagle Dogs

TO: Gary J. Burin, Toxicologist
SIS
Hazard Evaluation Division (TS-769)

FROM: John A. Quest, Toxicologist
Toxicology Branch
Hazard Evaluation Division (TS-769)

I was asked to review the attached dog study on Chlorpyrifos for use in a registration standard. The original draft was performed by Dynamac. However, for purposes of expediency I completed the review myself and therefore the document does not have the normal DER cover page.

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STUDY TYPE: Two-year dietary feeding studies in beagle dogs.

CITATION: McCollister SB, Kociba RJ, Gehring PJ, and Humiston CG. 1971. Results of two-year dietary feeding studies on Dowco® 179 in Beagle dogs: T35, 12-44793-18. (An unpublished report prepared by Dow Chemical, USA, Midland, MI).

ACCESSION NUMBER: 240192009.

MRID NUMBER: 00064933.

LABORATORY: Chemical Biology Research, Dow Chemical, USA.

TEST MATERIAL: Chlorpyrifos [0,0-Diethyl-0-(3,5,6-trichloro-2-pyridyl) phosphorothioate]; Dowco® 179, Lot No. CP 523-CD 235C; Purity: 98.8 percent (U.V.), 97.2 percent (GLC).

PROTOCOL: The study was conducted in two phases: Phase I in which the test compound was given for 1 year and then three months were allowed for recovery; Phase II in which compound feeding continued for 2 years.

PHASE I.

1. Eleven-month-old Beagle dogs were used.

2. Five groups (3 animals/sex/group) were fed Dowco® 179 in the diet at dosage levels of 0.01, 0.03, 0.1, 1.0 or 3.0 mg/kg/d for one year. A sixth group of animals served as controls. The composition of the control diet was not specified in the sponsors report.

3. The following parameters were evaluated: apearance and signs of increased cholinergic activity (daily); body weights in the one-year study were reported only at the end of the study; food consumption (weekly during months 1-3, and one week of each month thereafter), hematology* (prior to feeding and at 1, 2, 3, and 12 months on control animals and those which received 1.0 and 3.0 mg/kg/day); urinalysis* (pretest, at one month, and prior to sacrifice on control animals and those which received 1.0 and 3.0 mg/kg/day); plasma and RBC's cholinesterase activity (pretest, 1 and 2 weeks, and 1, 3, 6, 9, and 12 months on test in all dogs, six weeks after recovery on control and 0.1, 1.0 and 3.0 mg/kg/day groups, 3 months after recovery on control and 1.0 and 3.0 mg/kg/day groups);

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*Parameters studied included packed cell volume, hemoglobin, RBC count, WBC total and differential count, and prothrombin time.

brain cholinesterase activity (one dog/sex from each group at the end of the one year test period, and two dogs/sex from the control group and the 1.0 and 3.0 mg/kg/day groups at the end of 3 month recovery period); and other clinical chemistry studies ** (all dogs, pretest and at 1, 3, 6, and 12 months).

4. At the end of the one year period, one male and one female dog from each group were necropsied. The weights of heart, liver, kidneys, spleen, testes, and brain were determined.

5. Histopathological examination of 29 tissues from each animal in the control group, and groups that received 1.0 and 3.0 mg/kg/day was performed. The tissues examined were those required by the EPA Guidelines (1982) except for skin, thymus, salivary glands, caecum, rectum and gall bladder. All tissues were preserved in 10% formalin.

6. Students' "t" test was used to test for the significance of changes in cholinesterase activity.

Phase II.

1. Ten-month-old Beagle dogs were used.

2. Five groups (4 animals/sex/group) were fed Dowco® 179 in diet at dosage levels of 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day for two years; a sixth group of animals served as control. The composition of the control diet was not specified in the sponsor's report.

3. Body weights in the two-year study were reported at 6, 12, 18, and 24 months and not at weekly or biweekly intervals as stated in the protocol. Appearance of the animals, and food consumption were noted as in Phase I. The following parameters were evaluated in all animals in the control group and those which received 1.0 and 3.0 mg/kg/day: hematology*** (pretest, and at 1, 6, 12, 18, and 24 months); urinalysis*** (pretest, and at 1, 12, and 24 months); plasma and RBC's cholinesterase activity (pretest, at 1 week, and at 1, 3, 6, 12, 15, 18, and 24 months); brain cholinesterase activity (at 24 months); and other clinical chemistry studies*** (pretest and after 1 and 24 months in all dogs, and also in dogs in the 0, 1 and 3 mg/kg dose groups after 6, 12 and 18 months). Bromsulfalein retention was measured in all dogs (pretest and terminally), and on control dogs and those which received 1.0 and 3.0 mg/kg/day (at 12 months). All dogs were given complete physical exams including neurologic and ophthalmoscopic examination prior to termination.

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*Parameters studied included specific gravity, pH, sugar and albumen content, and microscopic examination of the sediment.

**Parameters included BUN, AP, SGOT, and SGPT.

***Parameters studied were the same as in Phase I.

4. All animals were sacrificed and necropsied at the end of two years and organ weights of the brain, heart, liver, kidneys, spleen, and testes were determined.

5. Histopathologic examination was performed on 29 tissues from each animal in the control group and those which received 3.0 mg/kg/day. The tissues examined and preserved were those specified for Phase I of the study.

RESULTS: The results obtained in this study were similar in Phase I and II; any differences observed between the two phases of the study are indicated below.

1. Appearance and Behavior: No data were presented. However, the authors stated that "no clinical signs of toxicity" and no "greater than normal cholinergic activity" were observed.

2. Body Weights: Male dogs that received the test compound for one year showed a slight increase in body weight gain (5-10 percent) over those of the control (4 percent). Females that received 0.03 mg/kg/day, showed a 4 percent body weight gain as compared to 9 percent in the control.

   Dogs which were used for the two-year study, showed an inconsistent gain in body weights. The percent increase in their mean body weights as compared to the initial mean body weight is shown in Table 1.

   TABLE 1. Body Weight Gain (Percent of Initial Body Weight) at the end of the 2-Year Study

Sex	Control	Dose (mg/kg/day) 0.01	0.03	0.1	1.0	3.0
Males	14	23	18	18	11	7
Females	25	17	19	25	14	19

   The smaller than control body weight gain of the males which received 3.0 mg/kg/d was also observed at 12 and 18 months.

3. Food Consumption: In the one-year study, cumulative mean food consumption for the 12 months was increased in males which received 0.1 and 1.0 mg/kg/day; the increase averaged 9.1 and 11.2 percent, respectively. Females in the two highest dose groups (1.0 and 3.0 mg/kg/day) showed a decrease in the same parameter averaged 12.6 percent and 9.4 percent of the control values, respectively. However, in both sexes, there was no dose-effect relationship.

No dose-related change in the cumulative mean food consumption of males in the two-year study was observed. Females of the smallest (0.01 mg/kg/d) and the highest (3.0 mg/kg/d) dose groups showed a decrease of 11.6 percent, and an increase of 13.6 percent in the cumulative mean food consumption, respectively.

4. Hematology and Urinalysis: No compound-related effects were found in the various hematologic parameters tested and in the urinalyses performed both one- and two-year studies.

5. Cholinesterase Activity:

°Plasma ChE activity, in the one-year study, was significantly decreased in all male and female dogs except the 0.01 mg/kg/day group. The effect was dose-dependent and appeared as early as 7 days in the treatment. The percentage decrease from control in plasma ChE activity averaged 11.2, 25.9, 45.1, 65.8 and 74.6 percent in male dogs receiving 0.01, 0.03, 0.1, 1.0 and 3.0 mg/kg/d of Dowco® 179, respectively. The decrease in the plasma ChE activity of females averaged 5.6, 20.9, 38.8, 63.8 and 71.0 percent of the control values at the above dosage levels, respectively. Cholinesterase activity returned to normal levels 14 days after cessation of test-chemical administration. The decrease in plasma ChE activity induced by the 0.1, 1.0 and 3.0 mg/kg/d dose levels in both male and female animals, was statistically significant at all test intervals; the decrease in the same parameter induced by the 0.03 mg/kg/d dose level was statistically significant at certain test intervals only; and the decrease observed at the 0.01 mg/kg/day dose level was not statistically significant. The NOEL was 0.01 mg/kg/day for male and female dogs.

Plasma cholinesterase activity in the 2-year study followed a similar pattern. In males, doses of 0.1, 1, and 3 mg/kg produced significant decreases in ChE activity (-40, -64 and -75%, respectively); the 0.03 mg/kg dose reduced ChE activity -19%; this was not a statistically significant effect but it may be of biological significance. In females, doses of 0.1, 1, and 3 mg/kg produced significant decreases in ChE activity (-30, -59, and -63%, respectively) whereas 0.03 mg/kg did not (-0.3%). The NOEL was 0.01 mg/kg for male and 0.03 mg/kg female dogs.

Red blood cell (RBC) ChE activity was significantly reduced only in animals that received 1 and 3 mg/kg for 1 year, males: (-51 and -66%, respectively; females: -49 and -71%, respectively). No significant changes occurred at doses of 0.01-0.1 mg/kg. RBC ChE activity returned to normal after 92 days in animals which received the 1 and 3 mg/kg doses. The NOEL was 0.1 mg/kg for males and females.

In the two-year study RBC ChE activity was also significantly decreased in male and female animals that received the 1.0 and 3.0 mg/kg/day dose levels (males: -54 and -73%, respectively; females: -54 and -71%, respectively); 0.01 to 0.1 dosage levels showed no significant effect. The NOEL was 0.1 mg/kg/day for males and females.

•Brain cholinesterase activity of animals maintained on Dowco® for one year was not markedly changed from the control group. <u>However, only one animal/sex/group was used to determine brain ChE activity</u>. Animals (2/sex/group) that received 1.0 and 3.0 mg/kg/day and which were sacrificed 3 months after recovery, however, showed a slight but not dose-related decrease in brain ChE activity.

In the two-year study, the decrease in brain ChE activity with reference to control levels averaged 1.5, 6.7, 8.3, 7.2, and 20.8% in 4 male dogs per group receiving 0.01, 0.03, 0.1, 1.0, and 3.0 mg/kg/day of the test material, respectively. In the same study, the percent change from control in brain ChE were +7.1, +2.8, +11.8, +5.8, and -19.4 in 4 female dogs per group receiving respectively the same doses as the males. These changes were not statistically significant.

5. <u>Other Clinical Chemistry Studies</u>: A slight increase in alkaline phosphatase, and a slight decrease in SGOT activity was noticed in animals that received 1.0 and 3.0 mg/kg/day Dowco 179 for 1 year. However, these changes were neither dose-related nor statistically significant. In the two-year study, changes in these parameters were minimal. At 6, 12, and 18 months the clinical chemistry parameters studies (AP, SGOT, SGPT, BSP) were evaluated only in groups that received 1.0 and 3.0 mg/kg/d dosage levels, no test-compound-related effect was observed.

6. <u>Organ Weights</u>: No dose-related changes were observed in the weights of heart, liver, kidneys, spleen, testes, or brain, nor in the organ weight/body ratio for animals sacrificed at the end of 1 year, or after a 3-month recovery period. However, only one animal/sex/group was sacrificed at the end of the 1 year period, the remaining two animals/sex/group were sacrificed after a 3-month recovery period.

Organ weights and organ/body weight ratios for animals sacrificed at the end of the two-year period showed changes which were not dose-related. However, although not dose-related, a generalized increase in the heart and liver weights of male animals at all dose levels were observed. The most noticeable change from control was an increase in both the average liver weight, and in the liver/body weight ratios for males that received 3 mg/kg/day of Dowco® 179. The male liver body weight ratios were 1.6 for the control group and 3.47 for the highest dose group. These changes were statistically significant ($p < 0.05$).

8. Gross Pathology: Both control and treated groups showed similar results on gross pathological examination.

9. Microscopic Examination: One dog from the 3 mg/kg/d group in the one-year study had subcutaneous histocytoma diagnosed from a biopsy during the first month of study. One dog that received 1 mg/kg/day showed an accumulation of inflammatory cells in renal tubules.

In the two-year study, only tissues from control dogs and those that received 3.0 mg/kg/day were examined microscopically. Of the four males in the dosed group, two animals showed pleocellular foci in the liver and two showed mineralized foci in renal medullary tubules. These same lesions, at similar incidences, were found in the 3.0 mg/kg/d dosed females and in control dogs of both sexes.

DISCUSSION:

Plasma and RBC cholinesterase activities were significantly decreased during the study in animals at dosages as low as 0.03 mg/kg/day. This effect appeared as early as 7 days in the study and was reversed in the plasma as early as 14 days in the recovery period. Only animals at the highest dose level showed apparent inhibition of brain cholinesterase activity. The overall NOEL for ChE activity was 0.01 mg/kg/day for male and female dogs.

The increase in liver weight and in liver/body weight ratios for male dogs that received 3 mg/kg/day of Dowco® 179 was not associated with histopathological changes. It seems that the liver is the target organ for Dowco® 179 toxicity, as demonstrated by the significant increase in male liver weight at 3 mg/kg/day. No other toxic effects than ChE inhibition and liver organ weight increase were demonstrated to be due to the administration of the test compound.

Dogs used in both phases of this study were eleven (Phase I) and ten (Phase II) months old at the initiation of the study, which exceeds the usual range of 4-6 months used in this type of study. Furthermore, discrepancies existed between the protocol and the actual study. For instance, while the protocol specified that body weights would be determined at a weekly interval during the first six months and biweekly thereafter, data presented included body weights at 12 months in the one-year study, and at 6, 12, 18, and 24 months in the two year study. Similar discrepancies were observed in food consumption, clinical chemistry and histopathology parameters.

Microscopic examination tissues was not performed on any female nor on 3 out of the 4 males that received 1 mg/kg/day, contrary to specifications in the protocol. Furthermore, one dog was diagnosed to have had subcutaneous histocytoma as early as one month in the study and should have been excluded from the study.

CONCLUSIONS

Under the conditions of this study, dietary administration of Dowco® 179 to Beagle dogs for two years induced the following effects: 1) an inhibition of plasma and RBC cholinesterase activity; 2) an increase of liver and liver/body weight ratios in male animals which received 3 mg/kg/day, the highest dose administered. The overall NOEL for Dowco® in this study is considered to be 0.01 mg/kg/day based on the inhibition of plasma cholinesterase in both sexes of dogs.

CORE CLASSIFICATION: Supplementary.

This classification is based on the following deficiencies:

1. Data was not available at the intervals specified in the protocol for several of the parameters that were monitored (e.g. body weight, ophthalmology, etc.).

2. Histology data was not presented for each of the individual tissues examined.

The classification may be upgraded if the deficiencies can be adequately corrected.

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